



Short communication

## Semen evaluation in the chamois of Abruzzi (*Rupicapra pyrenaica ornata*)

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Received 27 October 2005; received in revised form 1 February 2006; accepted 7 February 2006  
Available online 15 March 2006

### Abstract

The chamois of Abruzzi (*Rupicapra pyrenaica ornata*) has been classified as endangered by the World Union for Conservation.

The objective of this study was to analyze seasonal differences in the characteristics of various male reproductive organs and in semen quality. The study was conducted during 2004 in the reserve of Lama dei Peligni (Italy) on three chamois males aged between 2 and 5 years. Males were captured during March–May months and October–December months. Various testicular and scrotal measurements were taken and semen was collected using an electroejaculator. Sperm motility pattern was evaluated using computer assisted sperm analyzer, membrane integrity using differential staining and morphology with phase contrast microscopy. Testicular size, sperm motility membrane integrity and the percent of morphological normal spermatozoa were greater during October–December.

The authors concluded that sperm characteristics are strongly influenced by season and that semen collected in this period (breeding season) has suitable quality for manipulation and long-term preservation.

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**Keywords:** Chamois; Electroejaculation; Semen evaluation; Season

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## 1. Introduction

Since 1973 the trade of the chamois of Abruzzi was allowed by CITES only for scientific research programs. This species is classified as “EN = Endangered” in the “Red Data Book” of the World Union for Conservation (IUCN).

Scientific references on chamois concern only the ethological aspects, no data has been published on reproductive tract and sperm characteristics.

Some authors described chamois reproductive season in October–December and the length of gestation is about 23–27 weeks (Lovari, 1986; Lovari and Pellegrini, 1998).

Because it is important to improve the knowledge on reproductive male characteristics for preventing this species extinction, the objective of this study was to describe some testicular and sperm characteristics of chamois of Abruzzi and to find in which months semen quality was suitable for manipulation and long-term preservation.

## 2. Materials and methods

### 2.1. Extender

The collected semen was diluted with Tris (hydroxymethyl-aminoethane)–citric acid–glucose (TCG) obtained according to Aboagla and Terada (2003) with Tris (375.0 mM, Sigma–Aldrich S.r.l., Milan, Italy), citric acid (124.0 mM, Sigma–Aldrich S.r.l.) and glucose (41.0 mM, Sigma–Aldrich S.r.l.) and buffered to pH 7.0 and osmolarity 375 mOsm (Salomon and Ritar, 1982).

### 2.2. Animal captures and collection

The research, was carried out during 2004, in the Reserve of Lama dei Peligni (Chieti, Italy), extended for about 1700 ha, nine of these limited by an enclosure. Eleven animals are housed in the reserve: three adult males aged between 2 and 5 years (object of the study), four adult females and four kids born in 2003. Males were captured in two different periods: March–May (MM period) and October–December (OD period). Captures were performed by tele-injection as previously described (Cuomo et al., 2003).

Scrotal circumference and upper–inferior (UI), lateral–medial (LM) and cranial–caudal (CC) testicular diameters were measured. The seminal collection was obtained by electroejaculation and volume was measured in a conical sterile glass tube graduated with 0.1 ml optical visible intervals. Then, semen was diluted 1:100 with TCG and placed in the Equitainer<sup>®</sup> (Hamilton Thorne Biosciences, Beverly, MA, USA). The evaluation was performed at the laboratory within 2 h.

### 2.3. Semen evaluation

Semen concentration was evaluated by haemocytometer after dilution (1:400) with 0.05% formal saline.

Sperm motility was evaluated by computer assisted sperm analyzer (CASA) system IVOS version 12.2 (Hamilton Thorne Biosciences) at regular intervals of 3, 10 and 34 h after collection. This device identified motile sperm by their brightness and mean size. Sperm kinetic characteristics considered were motility (M), progressive motility (PM), and point-to-point curvilinear velocity (VCL).

Table 1

Means ( $\pm$ S.D.) of the scrotal and testicular measurements (cm) in March–May (MM) period and October–December (OD) period, respectively, considered non-breeding and breeding season

| Measurement             | March–May (MM) period |                 | October–December (OD) period |                 |
|-------------------------|-----------------------|-----------------|------------------------------|-----------------|
|                         | Right testicle        | Left testicle   | Right testicle               | Left testicle   |
| Upper–inferior diameter | 5.8 $\pm$ 0.4 b       | 5.3 $\pm$ 0.2 b | 7.2 $\pm$ 0.3 a              | 6.2 $\pm$ 0.2 a |
| Lateral–medial diameter | 3 $\pm$ 0.1 a         | 2.3 $\pm$ 0.2 a | 3.5 $\pm$ 0.2 a              | 2.5 $\pm$ 0.2 a |
| Cranial–caudal diameter | 4.7 $\pm$ 0.3 a       | 4.1 $\pm$ 0.1 b | 5.2 $\pm$ 0.1 a              | 4.8 $\pm$ 0.2 a |
| Epididymis tail         | 0.4 $\pm$ 0.2 a       | 0.4 $\pm$ 0.1 a | 0.5 $\pm$ 0.1 a              | 0.5 $\pm$ 0.1 a |
| Scrotal circumference   | 14.3 $\pm$ 1.2 b      |                 | 16.8 $\pm$ 0.5 a             |                 |

Different letters in the same column differ significantly ( $P \leq 0.05$ ).

Sperm plasmalemma integrity was referred to viability using SYBR-14 and propidium iodide (PI) (Live/Dead Sperm Viability Kit<sup>®</sup>, Molecular Probes, Eugene, OR, USA) as described by Garner and Johnson (1995). A semen aliquot (50  $\mu$ l) was mixed with 5  $\mu$ l of SYBR-14 (10  $\mu$ M) and 2.5  $\mu$ l of PI (120  $\mu$ M) and incubated at 38 °C for 15 min. Slide was executed by placing 5  $\mu$ l of stained semen with 1  $\mu$ l of glutaraldehyde solution (3%) and evaluated with an epifluorescence microscope (BX 51, Olympus Italia, Segrate, Milan, Italy), at 1000 $\times$  magnification. Green spermatozoa (SYBR-14) were considered live, these partially or completely red (PI) were considered dead. The percentage of plasmalemma-intact spermatozoa was determined on at least 200 spermatozoa.

Percentage of morphologic alterations was determined by analysis of sperm with normal morphology on two different smears prepared by liquid sample preserved in formal saline buffer (0.05%) using a phase contrast microscopy (1000 $\times$ ) and at least 200 cells were observed.

#### 2.4. Statistical analysis

Scrotal circumference and testicular diameters in MM and OD periods were compared by paired *t*-test and where considered significant with  $P \leq 0.05$ . Volume, concentration, M, PM, VCL, viability and morphologic alterations in March–May versus October–December periods were compared in the three subjects at 3, 10 and 34 h by Wilcoxon matched—pairs signed-ranks test (*t*-test for non-paired value) and by paired *t*-test. The results were considered significant with  $P \leq 0.05$ .

### 3. Results

Testicular diameters in MM and OD periods are reported in Table 1. Every animal presented a light asymmetry between right and left testis. The scrotal circumference was significantly different in MM than in OD periods, as like as UI diameters in both testicles and CC diameter in sx testicle ( $P \leq 0.05$ ) while the other diameters were no significant ( $P \geq 0.05$ ). During the MM period the occipital gland, with a diameter of about 1 cm, was only palpable, while during the OD period it was visible as a protuberance of about 3.5 cm in diameter. Semen color was yellowish during MM and white and lactescent during OD.

Sperm kinetic characteristics, viability and sperm morphologic alterations during MM and OD periods are reported in Table 2. The statistical analysis showed significant difference in MM

Table 2

Means ( $\pm$ S.D.) of seminal parameters calculated at 3, 10 and 34 h in March–May (MM) period and October–December (OD) period, respectively, considered non-breeding and breeding season

| Parameters                         | March–May (MM) period |                |                | October–December (OD) period |                |                |
|------------------------------------|-----------------------|----------------|----------------|------------------------------|----------------|----------------|
|                                    | 3                     | 10             | 34             | 3                            | 10             | 34             |
| Time post-collection (h)           |                       |                |                |                              |                |                |
| Volume (ml)                        | 1.1 $\pm$ 0.2         |                |                | 0.6 $\pm$ 0.1                |                |                |
| Concentration ( $\times 10^9$ /ml) | 1.9 $\pm$ 0.2         |                |                | 1.6 $\pm$ 0.1                |                |                |
| Total motility (%)                 | 10.2 $\pm$ 1.5        | 3.2 $\pm$ 1.4  | 0              | 78.5 $\pm$ 3.2               | 72.1 $\pm$ 2.7 | 53.7 $\pm$ 3.5 |
| Progressive motility (%)           | 1.7 $\pm$ 0.4         | 0              | 0              | 69.6 $\pm$ 1.7               | 61.2 $\pm$ 2.1 | 43.9 $\pm$ 2.9 |
| VCL ( $\mu$ m/s)                   | 32.2 $\pm$ 1.1        | 15.5 $\pm$ 2.2 | 0              | 92.1 $\pm$ 13                | 83.2 $\pm$ 8.9 | 65.4 $\pm$ 7.4 |
| Viability (%)                      | 3.5 $\pm$ 0.8         | 0              | 0              | 81.3 $\pm$ 1.8               | 73.4 $\pm$ 2.2 | 65.9 $\pm$ 1.5 |
| Morphologic alterations (%)        | 41.2 $\pm$ 1.3        | 42.9 $\pm$ 1.2 | 43.5 $\pm$ 1.4 | 3.2 $\pm$ 0.6                | 4.8 $\pm$ 0.8  | 5.2 $\pm$ 1.2  |

versus OD period for volume ( $P \leq 0.05$ ), M and viability ( $P \leq 0.01$ ), PM, VCL and morphologic alterations ( $P \leq 0.001$ ).

#### 4. Discussion

The limitations of this research were the scant number of valued subjects (3), the large extension of the protected area, the restricted number of captures (6) in comparison with the attempts.

According to Karagiannidis et al. (2000) a significant relationship between testicular diameters, ejaculate volume and breeding season exists in buck. This correlation evidenced that the animals in reproductive activity with repeated coupling showed a reduction in semen volume for ejaculate.

Other semen parameters values reported in bibliography in small ruminants were: volume 0.7–2 ml, concentration 2–5  $\times 10^9$  spermatozoa/ml, motility 70–90% and a sperm abnormalities percentage 5–15% (Mann and Lutwac-Mann, 1981; Chemineau, 1986; Chemineau et al., 1991; Roca et al., 1992; Prado et al., 2002).

Data reported in this study on chamois of Abruzzi semen characteristics were comparable. In fact, semen concentrations in chamois were 1.9  $\pm$  0.2 and 1.6  $\pm$  0.1  $\times 10^9$  spermatozoa/ml, respectively, in MM and OD periods.

Seminal quality parameters in chamois of Abruzzi during OD period showed a M of 78.5  $\pm$  3.2% and PM of 69.6  $\pm$  1.7% after 2 h maintenance in Equitainer<sup>®</sup> which was preserved at 44% after 34 h. The trend of viability and percentage of abnormal spermatozoa was related after 5 °C semen storage (56.1  $\pm$  7.1–32.5  $\pm$  4.8  $\mu$ m/s, 45.9  $\pm$  2.3–38.8  $\pm$  2.1  $\mu$ m/s, 81.3  $\pm$  1.8–65.9  $\pm$  1.5% and 3.2  $\pm$  0.6–5.2  $\pm$  1.2%, respectively). In MM period the values showed a poor quality of semen, but the decrease was comparable with other studies (Table 2). Value of VCL in OD period (92.1  $\pm$  13  $\mu$ m/s) was significantly greater than in MM period (32.2  $\pm$  1.1  $\mu$ m/s). Because some studies reported that VCL was strongly correlated with IVF rates (Holt et al., 1994; Chan et al., 1990), it could be suitable a semen collection for long-term semen preservation in October–December months.

In chamois there seems to be a clear seasonal influence on both semen production and characteristics (Table 2) as it is also reported in some goat breeds (Karagiannidis et al., 2000). In goats living in temperate conditions, intense sexual activity was observed between October and April (Delgadillo et al., 1991), while in subtropical areas breeding season seems to start earlier than in temperate areas (Delgadillo et al., 1999). On the contrary, Karagiannidis et al. (2000) reported a breeding season in late summer and autumn with high quantity (volume and concentration) and

quality (percentage of motile and progressive spermatozoa, percentage of abnormal spermatozoa). In the buck the breeding season in temperate areas seems to be different from the chamois. A possible hypothesis could be related to the chamois different sensitivity to photoperiod or other influences on melatonin release.

The studied periods (March–May and October–December) could represent non-breeding and breeding season, if compared with bibliography, but this observation must be confirmed with the analysis of reproductive characteristics during whole year.

## 5. Conclusion

Semen characteristics of chamois of Abruzzi are highly influenced by season. The satisfactory semen production to try cryopreservation programs was produced in October–December, but more investigations are needed in order to identify the exact breeding period. Frozen semen could be utilized in artificial insemination, for genetic studies, to increase genotypic variability and resolve the problem of rupicaprine species extinction.

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